Identification of terpenoids from *Withania somnifera* as a HIV-1 entry inhibitors that prevent gp120 binding to CD4 using *In Silico* Approach

Swapna Kandagatla[1], Estari Mamidala[2]*

[1,2]*Department of Zoology, Kakatiya University, Warangal-506009, Telangana, India*

Abstract

Human immunodeficiency virus (HIV-1), the most prevalent and pathogenic type of the virus, is what causes acquired immunodeficiency syndrome (AIDS).Approximately 30 anti-HIV-1 medications have been applied to the management of AIDS. However, effective anti HIV-1 agents with less side affect and high inhibition potency are still in demand.The objective of this study was to identify the potential terpenoid compounds from *Withania somnifera* plant that might be active as anti-HIV-1 gp120 by molecular dockingthat inhibits viral attachment and entry for the treatment of HIV/AIDS patients.In this investigation we have performed molecular docking, to develop a novel anti-HIV drug. We have screened 12 terpenoids from a medicinal plant *Withania somnifera*for its probabilistic binding with the active site of the HIV-1gp120 domain the major contributor to the onset of the disease.The docking results were evaluated based on free energies of binding (ΔG), and the results suggested withasomidienone, 2,3-didehydrosomnifericin, withasomniferol B, amyrin and 24,25-dihydrowithanolide D to be potent inhibitors of HIV-1gp120. These scores were comparable with the standard BMS-378806 drug. The docking molecular study has identified the possible potential terpenoids compounds from *Withania somnifera*plant that might be used for anti-HIV-1 treatment. Taken together, the data obtained suggest that these compounds may serve as promising scaffolds for the development of novel, highly potent and broad anti-HIV-1 therapeutics.

Keywords: Human immunodeficiency virus-1 gp120, Terpenoids, Molecular docking

1. Introduction

Human Immunodeficiency Virus (HIV) infection leads to the development of acquired immune deficiency syndrome (AIDS), which weakens the host's defences against life-threatening infections. By the end of 2021, there were 38.4 million HIV-positive persons living with AIDS worldwide, and 650,000 people were predicted to have died from the disease (WHO, 2022). Undoubtedly, the global health community continues to have major concerns about AIDS. HIV-1 infection and AIDS can still be prevented or treated by inhibiting the HIV-1 virus's initial entry event into host cells, which is a promising but difficult strategy. The trimeric envelope glycoprotein spike (Env), a component of the HIV-1 virus, and host cell receptors engage in protein–protein binding interactions, which lead to HIV infection and the development of AIDS (Jarrahpour et al, 2012). Three gp120 envelope glycoproteins and three gp41 transmembrane proteins make up each trimericEnv spike. Two sequential gp120 protein binding events, each accompanied by changes in Env shape, are the first steps in the HIV-1 viral entry process (Judith et al, 2012).

When gp120 attaches to the T-cell CD4 receptor that is tethered to the cell membrane, HIV becomes attached. After connecting to CD4, gp120 undergoes a conformational shift that exposes the transmembrane chemokine co-binding receptor's site (CCR5 or CXCR4). After gp41 binds to the co-receptor, it rearranges to

form a six-helix bundle that enters the host cell membrane, resulting in fusion and viral entry (Jarrahpour et al, 2012). The CD4 receptor on a host cell is where the HIV-1 gp120 interacts. Beginning with this, HIV enters the host cell by joining its viral membrane to the membrane of the host cell. Asn52, Gln33, Lys46, Lue44, and Gln25 residues of CD4 engage via hydrogen bonds with Ser365, Asp280, Gly366, and Asp474 residues of gp120, respectively. Phe43 (CD4) interacts hydrophobically with Val430, Met426, Trp427, Glu370, Asn425, Ile371, Gly473 (all from gp120), and Arg 59 (CD4) interacts hydrophobically with Val430 (gp120). With Arg 59 of CD4, the carboxylate group of gp120 Asp368 forms a double hydrogen bond. CD4's Pro48 interacts with gp120's Ser365, Arg469, and Asp457 residues (Kwong et al, 1998).

Trp112, Val255, Thr257, Glu370, Phe382, Tyr384, Trp427, and Met475 are the only main chains of gp120 and 375-377 that are predominantly hydrophobic among the residues that line the Phe43 cavity of CD4 (Kwong et al, 1998). They share the same degree of conservation as the submerged gp120 hydrophobic core. It turned out that the piperazine and BMS-378806 ring was crucial for the antiviral action. Due to its anti-HIV characteristics, regular contact at the Phe43 cavity's entrance, and widespread presence in several anti-HIV compounds with reported activity. It is crucial to keep in mind that replacing components in existing medications is a crucial tactic for providing more synthetically accessible compounds (Wang et al, 2009).

A treasure trove of diverse metabolites, including steroids, flavones, alkaloids, glycosides, saponins, tannins, and terpenoids, can be found in ashwagandha. Traditional medical systems have traditionally employed ashwagandha to treat a number of illnesses (Mishra et al, 2000). The hunt for novel potent antiviral medicines, like terpenes, has become necessary due to the emergence of viral infections. The bioactive terpenes found in a variety of plants have demonstrated varying degrees of antiviral activity. As a result, a few terpenoids have been chosen for molecular docking with the gp120 target's active site. Terpenoids demonstrated a variety of functions, including anti-inflammatory, anti-aging, cardioprotective, anti-carcinogenic, anti-inflammatory, and anti-oxidant action (Kuboyam et al, 2006).

In the present investigation, attempts have been made to explore the interaction of 12 terpenoids of *Withania somnifera*with the active site of HIV particularly encompassing the gp120 using.The docking was performed to understand the protein-ligand complex to identify pharmacological targets. This is the first report on *in silico* approach on figuring out the probable interactions of various terpenoids of *Withania somnifera*with HIV-1 gp120.

2. MATERIALS AND METHODS

2.1 Protein Target Selection and Preparation

The Protein Data Bank (PDB) (www.pdb.org/pdb) was used to extract the three-dimensional (3D) crystal structures of (PDB ID: 4DKR) (Figure-1).

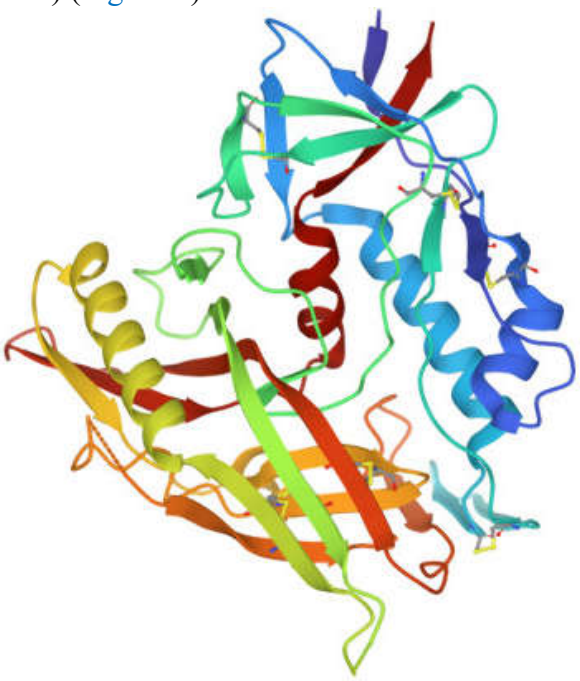

Figure-1. The molecular 3D modeling Crystal structure of HIV-1 gp120 (PDB ID: 4DKR) from RCSB PDB

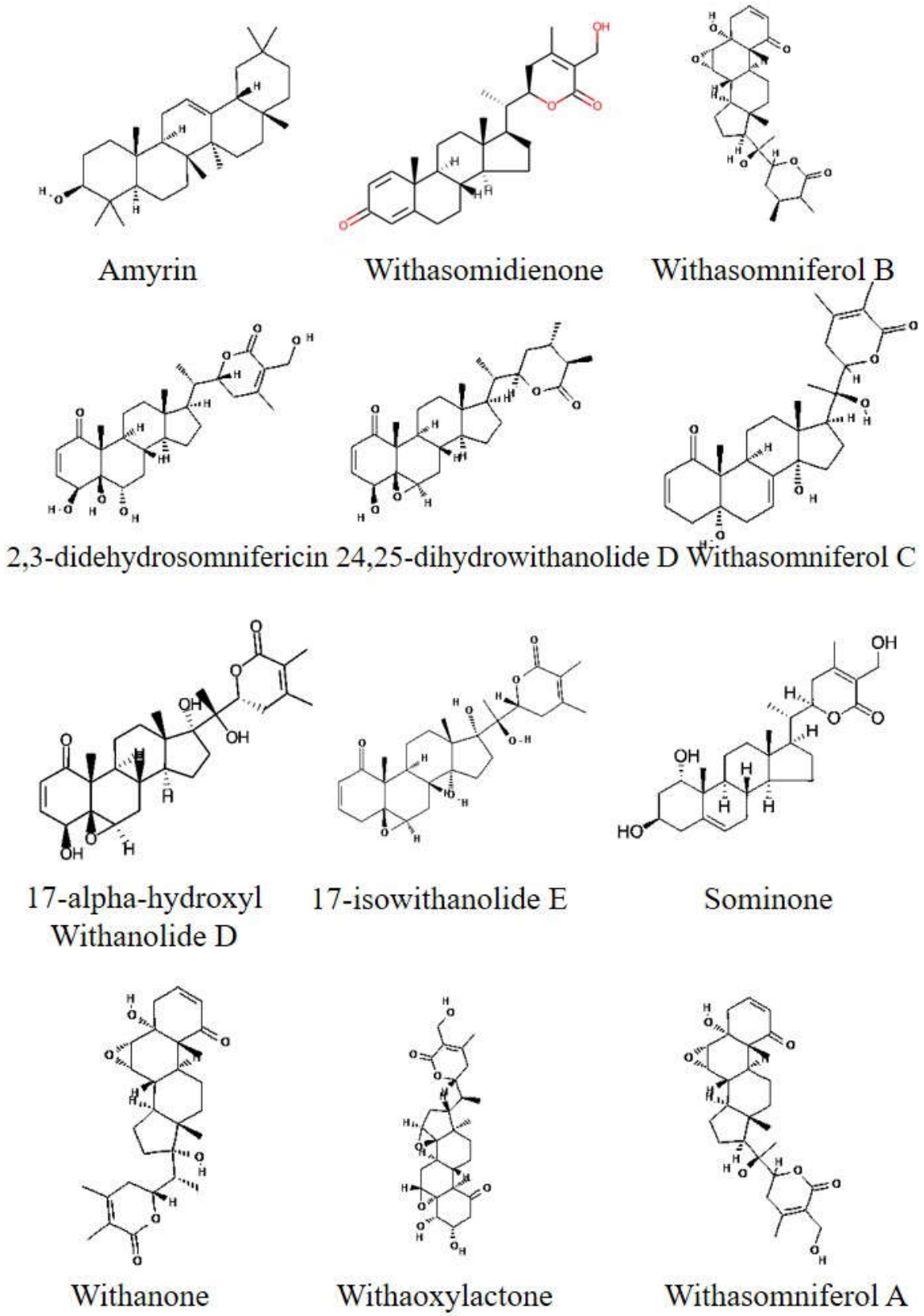

Figure-1. 2D chemical structures of selected terpenoids from *Withania somnifera*

PDB is a database that was created using Open Babel version 2.3.1 and contains information about experimental protein structures. Using BIOVIA Discovery Studio Visualizer, all heteroatoms, including co-crystallized ligand as well as water molecules and ions, were eliminated from the receptor during protein preparation. The target protein structure was given polar hydrogen atoms and Gasteiger charges, and the macromolecule was then recorded in PDBQT file format using ADT tools. In molecular docking investigations, the derived target protein structure was employed. These tools use various electrostatic, Vander Waal, hydrogen bonding, and desolvation phenomena to calculate binding-free energy (G). With the use of Auto Dock energy calculations, the stability of each docked pose was assessed.

2.2 Ligand Selections and Preparations

Three dimensional (3D) structures of the molecules were obtained in Simple Data File format (SDF) fromPub-Chem server (https://www.ncbi.nlm.nih.gov/pccompound) and were converted to PDB files. These small molecules were prepared for molecular docking by merging non-polar hydrogens, assigning Gasteiger charges, defining the rotatable bonds, assigning AutoDock type to each atom and saving them in PDBQT file format using AutoDock Tools (ADT) 1.5.6 (Mamidala et al, 2020). A total of twelve compounds from *Withania somnifera* were selected for the study, whileBMS-378806 were adopted as the control drug (Figure-2).

2.3 Molecular Docking Simulation

The AutoDock 1.5.6 was used to run molecular docking simulations. Assuming that macromolecules are stiff and that all the rotatable bonds in tiny molecules are rotatable. The grid maps and search grid were created using AutoGrid computations. ADT tool was used to construct the docking parameter file that contained instructions on how to move the ligand, use the appropriate map files, and use other properties. With the aid of Autodock tools, necessary hydrogen atoms, Kollman unified atom type charges, and solvation parameters were incorporated (Bhakta et al, 2012). Later, AutoDock employed grid and docking parameter files to execute the docking simulations. To carry out molecular docking, the Lamarckian Genetic Algorithm (LGA) with default parameters was used (Morris et al, 2009). 2,500,000 energy evaluations were applied to each of the 100 independent docking runs for small molecules.Lowest energy conformation of each small molecule was considered as active conformation among all the observed conformations and selected for analysis (Davella et al, 2022). Docking results were analyzed using Chimera X and Biovia Discovery Studio Visualizer.

3. RESULTS AND DISCUSSION

3.1 Molecular Docking Simulations

The result from the docking analysis of the reference inhibitor and bioactive terpenoids with the HIV-1 gp120 receptor (PDB ID: 4DKR) protein isshown in Table-1.The top 3terpenoidswith the highest binding affinity for the gp120 were further analyzed for binding interactions with HIV-1 gp120 complexed with human CD4.

3.2 Interaction of selected terpenoids with amino acids of target proteins

The amino acid interactions of the HIV-1 gp120 with reference inhibitor and plant derived terpenoids that demonstrated thehighest binding tendencies are represented in Table 1.The interacting residues of HIV-1 gp120 with respective ligand groups weremajorly through hydrophobic interactions and H-bonds. The gp120 range of binding affinity with all the 12 terpenoids shows from -4.86 to -8.85 Kcal/mol.

The docking analysis revealed that the reference inhibitor (BMS--378806) to the gp120 had binding energy -6.9 Kcal/mol. It was further observed that more than three terpenoids among 12, had higher binding affinity than the standard inhibitor used in this study. From the binding scores generated by the interacting terpenoids with the HIV-1 gp120, the top 3 docked

Table-1 Docking results of terpenoids from Withania *somnifera* with HIV-1 gp120

Ligand name (PubChem ID)	Binding energy (kcal/mol)	No. of Hydrogen bonds	Amino acid residues involving interactions	Hydrogen bond interactive amino acids
Amyrin (73145)	-6.68	-	-	-
Withasomidienone (000630)	-8.85	3	PRO:81, PRO:214, PRO:214, HIS:249, LYS:252, CYS:247, LYS:252 TYR:486	CYS:247, LYS:252 TYR:486
Withasomniferol B (101710596)	-7.89	2	PRO:212, ARG:379 and VAL:254	SER:447 ARG:379
2,3didehydrosomnifericin (70684083)	-7.69	3	PRO:81 PRO:214 ,PRO:214 , HIS:249	ASP:78 , LYS:252, ASN:262
24, 25-dihydrowithanolide D (23266166)	-6.61	2	PHE:53 and GLN:103	ASN:99 ,GLU:106
Withasomniferol C (101710597)	-6.23	3	PRO:81 , GLN:246 and HIS:249	GLU:83, CYS:247 , GLN:246
17-alpha-hydroxyl Withanolide D (23266161)	-5.11	5	PRO:364 , ARG:469	ASN:280, SER:365 ASP:457, GLY:458 GLY:366
17-isowithanolide E (21679024)	-4.86	3	Val:44	TRP:45 ,GLU:91 THR:90
Sominone (44249449)	-5.76	4	ASP:230 LYS:231 ASN:232 , PHE:233	ASP:230 ,ASN:241 LYS:231 ,THR:236
Withanone (21679027)	-6.35	1	LYS:202, 202 and 207	GLN:203
Withaoxylactone (101687981)	-5.05	1	VAL:68, GLN:114 ,ILE:208 , PRO:206	SER:209
Withasomniferol A (101710595)	-5.93	4	TRP:45	ASP:47 ,GLU:91 , ASN:92 ,LYS:487
Standard (BMS-378806)	-6.9	2	THR:51,GLN:103	LEU:52,GLU:106

terpenoids with the highest binding affinity are withasomidienone, withasomniferol B and 2,3-didehydrosomnifericin with corresponding binding energy of −8.85, -7.89 and −7.69 Kcal/mol respectively.

Except amyrin terpenoid, all the eleven terpenoids show different type of interactions with gp120. Withasomidienone exhibited several types of hydro-phobic interactions (Pi-Sigma, Pi-Pi T-Shaped, Pi-Alkyl, and Alkyl) with Pro81, Pro214, His249 and Lys252, a salt and attractive charges to Lys227 and His249 and hydrogen bond to Cys247, Lys252 and Tyr486. Three hydrogen bonds below $3.7A^0$ were observed between gp120 and withasomidienone (Figure-3).

Docking studies show this gp120 interacting through a donor H-bond between the nitrogen atom of the Withasomniferol B and the Ser447 residue, and acceptor H-bond between the carbonyl group and the Arg379 residue which is near to CD4 binding site of gp120. Withasomniferol B interacted via hydrophobic interactions to Pro212, Val254 and Arg379. A salt bridges interaction was also observed with Arg379 of gp120 (Figure-4).

2,3-didehydrosomnifericin interacted via H-bond to Asp78, Lys252 and Asn262. Hydrophobic interactions was observed with the Pro81, Pro214, Pro214 and His249 residues (Figure-5). Only two hydrogen bonds was formed with residues Leu52 and Glu106 of gp120 with standard drug BMS-378806. When compared to this standard drug, the selected terpenoid shows strong interactions with gp120.

Finally, it is anticipated that inhibitors targeting gp120 will be more successful in preventing HIV infection than those targeting gp41 because gp120 binding sites to CD4 are exposed in their original state, whereas gp41 binding sites are exposed after the gp120-CD4 complex has been established (Lombardi et al, 1996).The first effective step toward creating a grid that would distinguish between ligands fitting the cavity and those with an unsuitable shape was protein creation using both PDB data using the protein preparation wizard in Glide.

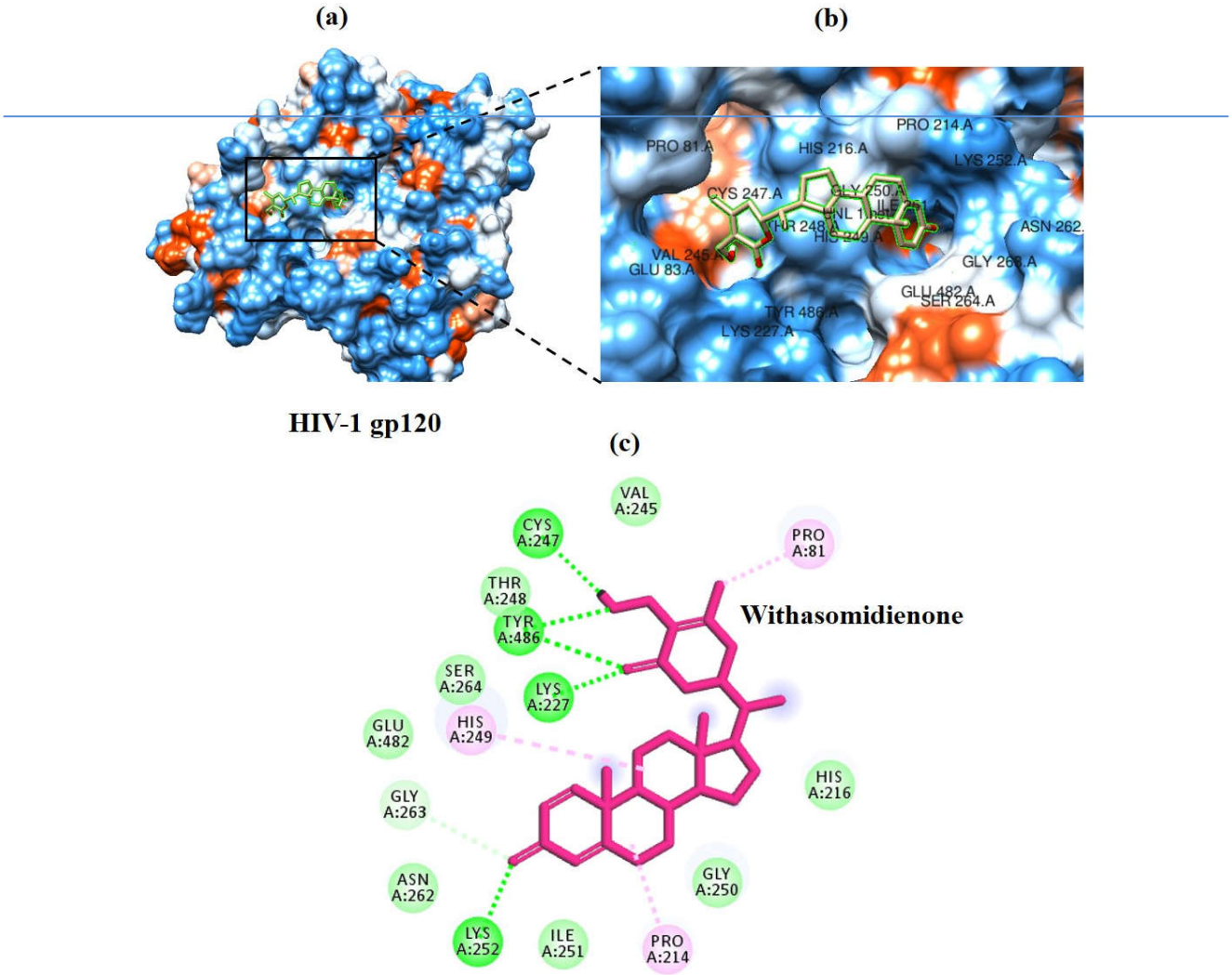

Figure-3. Withasomidienone terpenoid interactions with gp120.

(a) View of the hydrophobic binding pocket of gp120 in surface mode accommodating Withasomidienone (green) visualized by using Chimera X.

(b) Space filled view of ligand biding site of HIV-1 gp120 with docking ligand Withasomidienone

(c) Two-dimensional schematic representation of the binding mode of gp120 with withasomidienone. The residues of gp120 forming hydrogen bonds (green dotted lines) and van der waals contacts (pink dotted lines) with withasomidienone

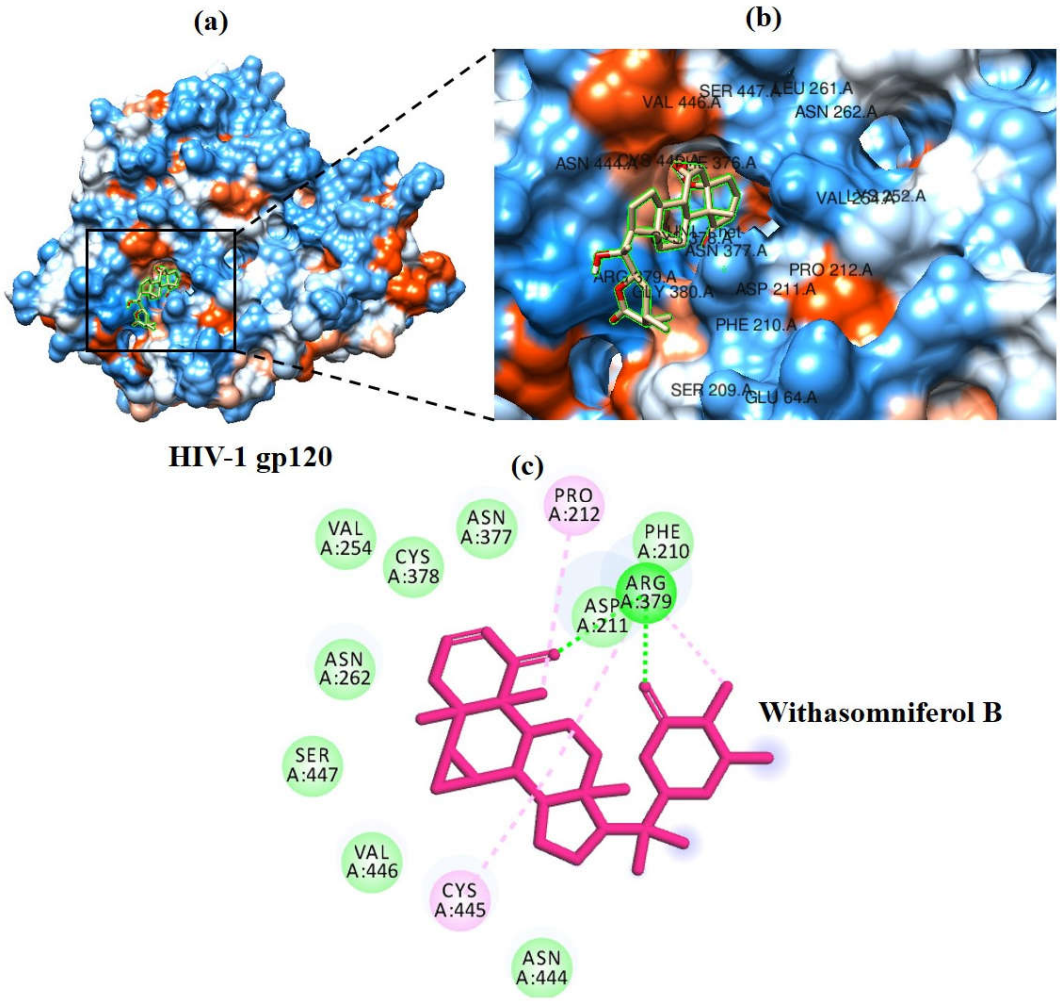

Figure-4. Withasomniferol B terpenoid interactions with gp120.

(a) View of the hydrophobic binding pocket of gp120 in surface mode accommodating Withsomniferol B (green) visualized by using Chimera X.

(b) Space filled view of ligand biding site of HIV-1 gp120 with docking ligand Withasomniferol B

(c) Two-dimensional schematic representation of the binding mode of gp120 with Withasomniferol B. The residues of gp120 forming hydrogen bonds (green dotted lines) and van der waals contacts (pink dotted lines) with Withasomniferol B

Gp120/gp41 envelope proteins have a particular affinity for CD4 receptors anchored in CD4+ T cells (Chen et al, 2012). The CC or CXC chemokine receptors, which are found on the surface of lymphocytes and monocyte/macrophages, signal after this contact. The HIV-1 envelope undergoes structural changes as a result of these contacts, which ultimately result in membrane fusion less than an hour after the initial HIV-T cell contact (Kwon et al, 2012).

The bridging sheet and the depression or cavity created at the junction of the outer and inner domains are where the CD4 co-receptor attaches. Complementary electrostatic potentials hold the interaction between the two proteins. In CD4 and gp120, the connections between 22 and 26 residues are surrounded by 219 van der Waals

interactions and 12 hydrogen bonds, respectively (Kwon et al, 2012). According to this study, the terpenoid withasomidienone was able to connect to the gp120 active pocket through three hydrogen bonds as well as additional hydrophobic bonding, and hydrophobic interaction. The conclusion drawn from the docking analysis is that Withasomidienone, 2,3-didehydrosomnifericin, Withasomniferol B, amyrin and 24,25-dihydrowithanolide D have the

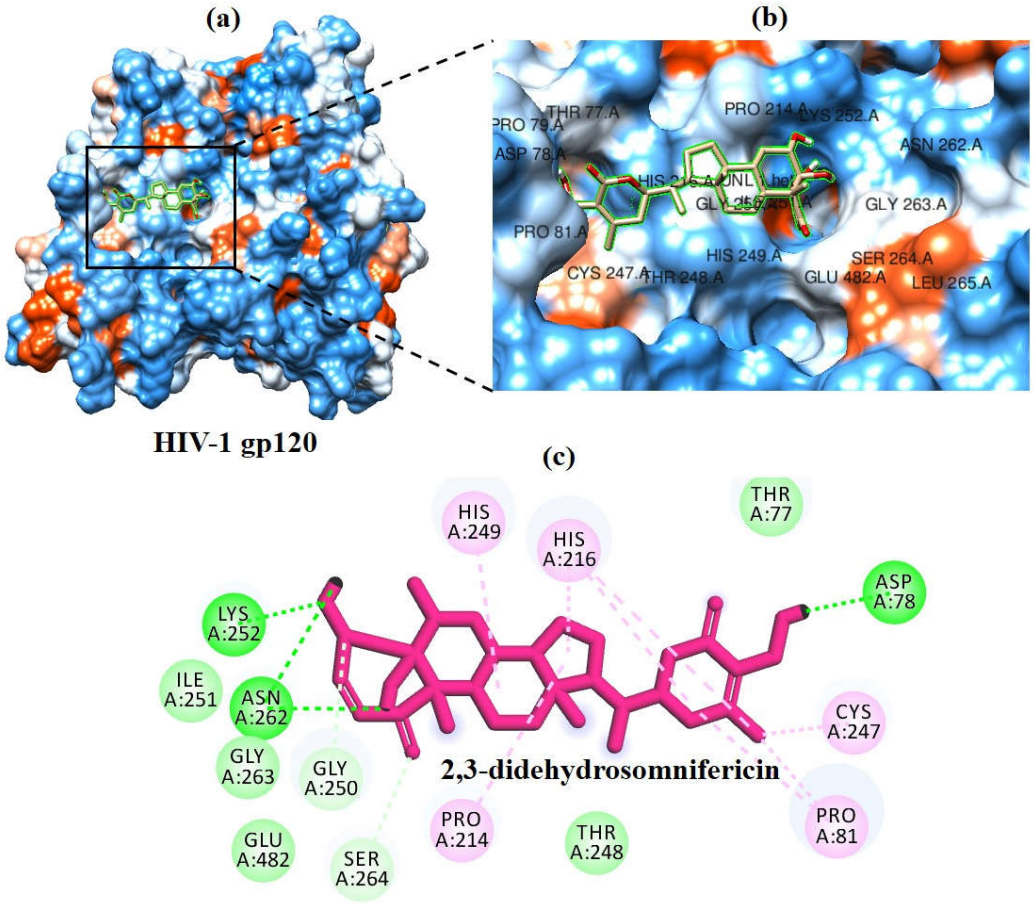

Figure-5. 2,3-didehydrosomnifericin terpenoid interactions with gp120.

(a) View of the hydrophobic binding pocket of gp120 in surface mode accommodating 2,3-didehydrosomnifericin (green) visualized by using Chimera X.

(b) Space filled view of ligand biding site of HIV-1 gp120 with docking ligand 2,3-didehydrosomnifericin

(c) Two-dimensional schematic representation of the binding mode of gp120 with 2,3-didehydrosomnifericin. The residues of gp120 forming hydrogen bonds (green dotted lines) and van der waals contacts (pink dotted lines) with 2,3-didehydrosomnifericin

interactions.

CONCLUSIONS

In the present study, the molecular docking is performed to explore the possible binding mode of HIV-1gp120 with 12 terpenoids of *Withania somnifera.*The best ligand conformation is chosen based on binding free energy value, hydrogen highest binding affinity with HIV-1gp120 when compare to standard drug. The docking molecular study has identified the possible potential terpenoids compounds from *Withania somnifera* plant that might be used for anti-HIV-1 treatment. Our study is perhaps the first to attempt inferring that terpenoids is a potential anti-HIV-1gp120 drug. Hopefully, the proposed molecule can be

put forward for a constructive concept of designing HIV inhibitors. Thus, it might be a useful candidate for HIV therapy.

Declaration of competing interest

The authors declared no conflict of interest

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